"only partially." The RIPK knockout mutant shows a reproducible reduction in RIN4 phosphorylation in response to AvrB, but no reduction in response to AvrRpm1, as assessed with an anti-phospho-T166 antibody. Consistent with this result, growth of a *P. syringae* strain expressing AvrB is only slightly enhanced in the *ripK* knockout, and the number of cells undergoing RPM1-induced cell death in response to this strain is slightly reduced. The authors speculate that the likely reason for these relatively modest effects is functional redundancy with other RLCK family members. Unfortunately, the large number of similar family members precludes an easy test of this hypothesis.

Another puzzling result is that AvrB and RIPK appear to compete for the same binding site on RIN4; when all three proteins are coexpressed, only AvrB immunoprecipitates with RIN4. It thus remains a possibility that AvrB phosphorylates RIN4 directly. Since the authors also show that RIPK can phosphorylate AvrB, one plausible model is that RIPK functions to phosphorylate AvrB, which then activates AvrB kinase activity (Figure 1B). The reduced phosphorylation of RIN4 in the *ripK* mutant background would then be the result of reduced AvrB activation.

Regardless of which model ultimately proves correct, the work of Chung et al. and Liu et al. have clearly established that NLR proteins can be activated by phosphorylation of other associated host proteins. It will be of interest to see how widespread this mechanism is among other plant and animal NLRs. At a more mechanistic level, the interesting question now becomes how phosphorylation of RIN4 alters its interaction with RPM1, and how this change leads to activation of RPM1.

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Evicting the Pneumococcus from Its Nasopharyngeal Lodgings

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Nasopharyngeal colonization by *Streptococcus pneumoniae* precedes invasive disease and mediates community transmission of the pathogen. In this issue, Moffitt et al. (2011) used proteomic analysis to identify conserved pneumococcal protein vaccine antigens that elicit T_H17-dependent responses capable of preventing such colonization.

Streptococcus pneumoniae (the pneumococcus) is one of the foremost bacterial pathogens in humans, causing massive global morbidity and more than a million deaths each year. However, it is essentially a commensal organism, asymptomatically colonizing the nasopharynx of a significant proportion of the human population, particularly young children and the elderly. Such carriage acts as a reservoir for transmission of the organism in the community, as well as a beachhead for subsequent penetration to otherwise sterile sites, including the middle ear cavity, lungs, blood, and brain, resulting in pneumococcal disease.

Development of effective and affordable pneumococcal vaccines has been a global health priority for many decades. Vaccines comprising purified capsular polysaccharides, the dominant pneumococcal surface antigens, were licensed in the late 1970s. However, these suffered from the shortcoming of poor immunogenicity in high-risk groups (particularly young children) because the component polysaccharides are T cell-independent antigens. Furthermore, the vaccine formulations (initially 14- and later 23-valent) provided incomplete coverage of the more than 90 known capsular serotypes of *S. pneumoniae*.

The poor immunogenicity of the polysaccharide antigens has been overcome by conjugation to protein carriers to elicit boostable, T cell-dependent immune responses. These conjugate vaccines are highly immunogenic in infants, and a 7-valent formulation covering the most important pediatric serotypes was first licensed in the late 1990s; it has since been incorporated into the routine childhood immunization programs in a number of countries. This vaccine has been spectacularly successful in reducing the incidence of invasive pneumococcal disease due to included serotypes in the target population. Moreover, the vaccine resulted in a massive reduction in asymptomatic nasopharyngeal carriage of these serotypes and a concomitant significant "herd immunity" effect in nonvaccinees (Centers for Disease Control, 2005). However, overall efficacy has been limited by the fact that the geographic distribution of disease-causing serotypes of *S. pneumoniae* is not uniform, nor is it static. The 7-valent vaccine was formulated with reference to the commonest pediatric serotypes in North America (covering 85%–90% of invasive infections), but coverage is significantly lower in other important geographic regions, including populations with a high pneumococcal disease burden. Superimposed on this are temporal changes in serotype prevalence induced by the vaccine itself. The high anticapsular antibody levels elicited by the vaccine are capable of blocking establishment of nasopharyngeal colonization by a vaccine-type pneumococcus. However, the vacated niche is being increasingly occupied by nonvaccine type pneumococci (so-called replacement carriage). Serotype replacement in the nasopharynx inevitably leads to serotype replacement in pneumococcal disease, as has been observed in a number of studies (Hicks et al., 2007).

The impact of widespread use of conjugate vaccines on the complex biology of pneumococcal disease is difficult to predict. The full effect of the vaccine on serotype prevalence may take many years to become apparent and will vary from region to region depending upon levels of endemic carriage, baseline seroprevalence, and rates of vaccine utilization. Reformulation of conjugate vaccines to account for geographic differences and vaccine-driven changes in serotype prevalence may be necessary (10- and 13-valent formulations have recently been licensed), but there are cost implications, as well as a limit to the number of serotypes that could ever be accommodated.

In this issue of *Cell Host & Microbe*, Moffitt et al. (2011) describe a major step toward development of a vaccine that prevents colonization by all pneumococci regardless of serotype, bypassing the serotype replacement issue altogether. This study was built on earlier work from their laboratory aimed at understanding the mechanism whereby humans develop age-related natural resistance to pneumococcal carriage, a process that did not correlate with acquisition of anticapsular antibodies (Lipsitch et al., 2005). They subsequently demonstrated that mucosal immunization of mice with killed, nonencapsulated pneumococci or a mixture of purified protein antigens protected them from colonization with *S. pneumoniae* in an antibody-independent fashion (Malley et al., 2005; Basset et al., 2007); this protective effect was dependent on interleukin (IL)-17A-producing CD4+ T cells (T_H17 cells) (Lu et al., 2008). Immunization did not prevent establishment of colonization like the conjugate vaccine but rather it promoted clearance of colonizing pneumococci by recruitment of neutrophils to the nasopharyngeal mucosa.

Moffitt et al. (2011) have now screened an *E. coli* expression library (comprising over 2000 clones) representing >96% of the proteins encoded by the pneumococcal genome for antigens that elicit the strongest T_H17 responses. This involved pulsing murine peritoneal macrophages with pools of clones, followed by incubation with CD4+ T cells harvested from mice that had been immunized with their whole-cell vaccine. Culture supernatants were subsequently assayed for IL-17A to identify those antigen pools that activated T_H17 cells. Clones from positive pools were then tested individually. The amino acid sequences of the proteins expressed by each of the 100 positive clones were then subjected to bioinformatic analysis to prioritize candidate antigens (those >100 aa in length with >90% identity in all of the 22 sequenced *S. pneumoniae* genomes, without homology to any human proteins, and with <40% identity to other bacterial proteins).

Five of the seventeen antigens that passed this screen were then selected on the basis of high expression in recombinant *E. coli* and successfully purified. Two of these, SP0148 and SP2108 (the solute-binding components of ABC transport systems specific for aromatic amino acids and maltose, respectively), induced IL-17A secretion when used to stimulate splenocytes from three different mouse strains that had been infected intranasally with live *S. pneumoniae*. Furthermore, separate stimulation of peripheral blood mononuclear cells from several healthy human donors with the same two antigens also resulted in IL-17A secretion, indicating that antigen-specific T_H17 cells are primed during natural exposure of humans to pneumococci. Thus, in humans and mice, the antigens are accessible to antigen-presenting cells during mucosal colonization and processed for presentation to CD4+ T cells. Finally, Moffitt et al. (2011) demonstrated that mucosal immunization with either SP0148 or SP2108 conferred highly significant protection against colonization with *S. pneumoniae* in two separate strains of mice. Protection required both CD4+ T cells and IL-17A, as treatment of immunized mice with anti-CD4 or anti-IL-17A before and after challenge abrogated protection. Other proteins identified in the screen that elicited slightly weaker IL-17A responses than SP0148 or SP2108 did not confer significant protection against colonization, nor did mucosal immunization with StkP, another pneumococcal surface protein known to elicit antibody-dependent protection against invasive disease after parenteral immunization.

The findings of Moffitt et al. (2011) are significant for several reasons. First, they show that it may be possible to almost completely prevent nasopharyngeal carriage of all pneumococci, regardless of serotype, by mucosal immunization with just a handful of purified protein antigens. Such a vaccine could have a major and sustained impact on global morbidity and mortality from invasive pneumococcal disease, as colonization almost invariably precedes invasive disease. Moreover, because asymptomatic carriers are the major reservoir for transmission of pneumococci in the community, a substantial herd immunity effect would be predicted. A potential caveat, however, is whether complete eviction of

S. pneumoniae might provide opportunities for other potential pathogens that normally compete with the pneumococcus for the nasopharyngeal niche. A second significant finding is that although many studies have demonstrated that parenteral immunization with other conserved pneumococcal proteins can elicit antibody-dependent protection against invasive disease (summarized by Paton, 2011), these proteins were not among those identified in the T_H17 screen. This indicates substantial nonoverlap between sets of antigens recognized by protective antibodies and T_H17 cells. Third, the screening approach described by Moffitt et al. (2011) is directly applicable to identification of potentially protective T_H17-dependent antigens displayed by other mucosal pathogens, such as nontypable *Haemophilus influenzae*, *Neisseria meningitidis*, *Staphylococcus aureus*, *Bordetella pertussis*, and *Mycobacterium tuberculosis*.

In the above studies, T_H17-dependent protection against pneumococcal colonization elicited by mucosal immunization required coadministration with cholera toxin, a strong mucosal adjuvant. Although nontoxic mucosal adjuvants are available, there are still regulatory hurdles to be overcome as far as human use is concerned. In this context, a recent study has shown that subcutaneous immunization with whole-cell vaccine, using an aluminum hydroxide adjuvant formulation approved for human use, also elicited strong T_H17-dependent protection against pneumococcal colonization. Significantly, however, it also elicited antibody-dependent protection against fatal sepsis (Lu et al., 2010). This raises the prospect of a single, injectable alum-adjuvanted vaccine formulation combining the purified proteins that elicit the best T_H17 responses to prevent carriage (i.e., SP0148 and SP2108) with other pneumococcal proteins, such as pneumolysin, CbpA, and PspA, known to elicit strong antibody-dependent protection against invasive disease (Ogunniyi et al., 2007). Such a vaccine would provide two distinct lines of defense against this formidable pathogen.

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